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Dehalogenation of polychlorinated biphenyls and polybrominated diphenyl ethers using a hybrid bioinorganic catalyst†

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The environmentally prevalent polybrominated diphenyl ether (PBDE) #47 and polychlorinated biphenyls (PCBs) #28 and #118 were challenged for 24 hours with a novel biomass-supported Pd catalyst (Bio-Pd^0). Analysis of the products *via* GC-MS revealed the Bio-Pd^0 to cause the challenged compounds to undergo stepwise dehalogenation with preferential loss of the least sterically hindered halogen atom. A mass balance for PCB #28 showed that it is degraded to three dichlorobiphenyls (33.9%), two monochlorobiphenyls (12%), and biphenyl (30.7%). The remaining mass was starting material. In contrast, while PCB #118 underwent degradation to yield five tetra- and five trichlorinated biphenyls, no less chlorinated products or biphenyl were detected, and the total mass of degraded products was 0.3%. Although the Bio-Pd^0 material was developed for treatment of PCBs, a mass balance for PBDE #47 showed that the biocatalyst could prove a potentially useful method for treatment of PBDEs. Specifically, 10% of PBDE #47 was converted to identifiable lower brominated congeners, predominantly the tribrominated PBDE #17 and the dibrominated PBDE #4, 75% remained intact, while 15% of the starting mass was unaccounted for.

Introduction

Polychlorinated biphenyls (PCBs) have found widespread use in a diverse range of applications, with around 1.2 million tonnes produced worldwide, of which approximately 67 000 and 40 000 t were produced and used, respectively, in the UK.[1] Owing to concerns about their adverse effects on humans and wildlife,[2] their production—but not their use—ceased in the UK and throughout most of the industrialised world in the late 1970s. Although UK human exposure to dioxins and dioxin-like PCBs *via* the diet has fallen in recent years in response to the cessation of their production,[3,4] concentrations of non-dioxin-like PCBs in indoor air in the West Midlands conurbation of the UK have shown no significant decline between 1997 and 2004,[5] and the most recent survey of dietary exposure of the UK population showed a substantial proportion of schoolchildren and toddlers to be exposed at levels exceeding the UK government's recommended tolerable daily intake of dioxins and dioxin-like PCBs.[4]

Polybrominated diphenyl ethers (PBDEs) have been used widely as flame retardants. In recent years, the production and use of PBDEs has been in the guise of three formulations: penta (consisting primarily of BDEs 47 and 99, 37% each, alongside smaller amounts of other tetra-, penta- and hexa-BDEs), octa (a mixture of hexa (10–12%), hepta- (44–46%), octa- (33–35%), and nona- (10–11%), and deca (98% decabromodiphenyl ether—BDE 209—and 2% various nona-BDEs).[6,7] Global production has been dominated by the deca commercial formulation, with worldwide demand in 2003 an estimated 56 418 t.[8] This is down from the 2002 estimate of 65 677 t, but in line with estimates for 2001 and 1999 of 56 150 t and 54 800 t, respectively.[8] By comparison, the 2001 global demand for the penta-product was 7500 t,[8] down slightly from 8500 t in 1999.[9] Production and use of commercial PBDE formulations in Europe was considerably less than in North America: for example, in 2001, 7100 t of penta-product were used in North America, compared with just 150 t in Europe.[8] The uses for these commercial formulations are myriad: the penta-product was employed principally to flame retard polyurethane foams in carpet underlay, vehicle interiors, furniture and bedding; the octa-formulation was used to flame retard thermoplastics such as high impact polystyrene; and the deca-product is used principally in plastic housings for electrical goods like TVs and computers, as well as textiles.[6] As a result of concerns surrounding these contaminants, owing to their presence in the diet and in indoor air and dust,[5,9–11] and human tissues,[12] coupled with evidence relating to their potential adverse effects on human health,[7,13] several jurisdictions have banned the marketing and use of penta- and octa-BDEs. Furthermore, the main U.S. producer, together with the U.S. EPA, have reached a voluntary agreement to discontinue production of the penta- and octa-BDE mixtures.

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The above observations of elevated indoor concentrations of these compounds are consistent with a continuing legacy reservoir of in-use PBDEs and PCBs which requires management to limit their impact on current exposure and ultimate disposal if they are not to provide a substantial source of future human exposure to these compounds. This may occur *via* direct inhalation and ingestion of contaminated indoor air and dust, and in time *via* dietary exposure following their emission, transport and incorporation into the food chain.[14] Environmentally-benign disposal of this reservoir of PCB and PBDE-containing material, both in waste streams and in-use, therefore presents a significant challenge. Some sulfate-reducing bacteria have the capacity to reductively dehalogenate chlorinated aromatic compounds by a mechanism(s) that is still not yet fully defined.[15,16] However, it is reported that the biodegradation of polychlorinated biphenyls (PCBs) in the environment is slow.[17] Furthermore, the use of biological systems for the treatment of industrial wastes has several drawbacks, *e.g.*, biological systems are sensitive to alterations in pH, temperature, ionic strength, solvents or other parameters, and biosystems usually require physiologically compatible conditions. A potentially more robust alternative to reductive dehalogenation of halogenated aromatic compounds is the use of palladized cells of sulfate-reducing bacteria. A particularly environmentally beneficial aspect of such a biomass supported Pd catalyst is that it can be manufactured from waste.[18,19] We have shown previously that such a Bio-Pd^0 hybrid material is effective in the reductive dehalogenation of PCBs.[20] However, the previous analysis relied upon measurement of liberated chloride ion alone and hence detailed information on the breakdown products or the degradation pathway was not given. This paper therefore reports on the breakdown products resulting from Bio-Pd^0 treatment of two of the most prevalent PCBs (congeners #118 and #28) and also on the potential of the Bio-Pd^0 material to reductively dehalogenate PBDE #47, one of the two principal congeners present in the widely used penta-BDE formulation.

Experimental

Organism, and preparation of Pd catalysts

Desulfovibrio desulfuricans ATCC 29577 was cultured as described previously.[21] For the preparation of palladised biomass (Bio-Pd^0) a known volume of a 2 mM solution of Na_2PdCl_4 (to pH 2.0 with 0.1 M HNO_3) was placed in 50 mL butyl rubber sealed serum bottles and de-gassed with oxygen free nitrogen (OFN: 15–20 min). A mid-logarithmic phase cell suspension of *Desulfovibrio desulfuricans* was added to a final ratio (wt of Pd:dry wt of cells) of 1:3 *via* a syringe under OFN. The cell suspension was left for 1 hour (30 °C) to allow initial biosorption of Pd(II) to the biomass surface. Hydrogen was then sparged into the suspension (15 min) to fix the palladium onto the cells as Pd^0 (identified as palladium metal by X-ray powder diffraction analysis[21]) *via* cellular hydrogenase activity.[21] The suspension was left to stand (15 min) allowing the Pd-coated biomass to fall under gravity, and the recovered Bio-Pd^0 was washed three times in distilled water and once in acetone (10 ml) several times, dried in air to constant weight, and ground.

Dehalogenation tests

The following individual compounds were separately challenged with Bio-Pd^0: PCBs #118 and #28, and PBDE #47 (10 μg of compound) (Table 1). The Bio-Pd^0 (2 mg + 0.2 mg) was placed into 10 mL serum bottles. The target compound was then added (10 μg) as a suspension in a carrier of 20 mM MOPS–NaOH buffer pH 7.0 (8.8 mL). The bottles were sparged with oxygen-free nitrogen (15 min). The reaction was started by the addition of 1 mL of freshly prepared 1 M formate (final volume of reaction mixture: 10 mL). The formate is split catalytically by the Pd^0 to give hydrogen as the reductant for the reductive dehalogenation reaction *via* the Pd^0 catalyst. The use of formate is preferable to hydrogen gas as its concentration in solution can be accurately known. After 24 h exposure the experiment was stopped *via* removal of the

Table 1 Percentage yields of products identified after 24 h following challenge of PCB #118, PCB #28 and PBDE #47[a] with Bio-Pd^0

Congener	Yield (%)	Congener	Yield (%)	Congener	Yield (%)
2,3′,4,4′,5 (PCB #118, starting product)	>99%	2,4,4′ (PCB #28, starting product)	30.9	2,2′,4,4′ (BDE #47, starting product)	66.0 (83.0)
Tetrachlorobiphenyls		Dichlorobiphenyls		Tribromodiphenyl ethers	
2,3′,4,5 (PCB #67)	0.16	4,4′ (PCB #15)	3.8	2,4,4′ (BDE #28)	0.4 (0.6)
2,4,4′,5 (PCB #74)	0.06	2,4′ (PCB #8)	15.7	2,2′,4 (BDE #17)	5.9 (5.4)
2,3′,4′,5 (PCB #70)	0.03	2,4 (PCB #7)	14.4		
2,3′,4,4′ (PCB #66)	0.01				
3,3′,4,4′ (PCB #77)	0.004				
Trichlorobiphenyls		Monochlorobiphenyls		Dibromodiphenyl ethers	
2,4,5 (PCB #29)	0.040	2 (PCB #1)	7.5	2,4 (BDE #7)	0.1 (0.2)
2,3′,5 (PCB #26)	0.002	4 (PCB #3)	4.5	2,4′ (BDE #8)	0.2 (0.2)
2,4′,5 (PCB #31)	0.005			2,2′ (BDE #4)	1.7 (3.1)
2,3′,4′ (PCB #33)	0.008	Biphenyl	30.7	4,4′ (BDE #15)	0.2 (0.0)
2,3′,4 (PCB #25)	0.001				
				2-Monobromodiphenyl ether (BDE #1)	0.0 (1.6)
Total	~100	Total	107.4	Total	74.5 (93.9)

[a] Data shown are values obtained for the duplicate runs for this starting compound only.

aqueous supernatant, which was immediately partitioned against hexane (2 mL). The Bio-Pd^0 mass was removed and extracted with hexane (2 mL). The hexane extracts were then subjected to analysis.

Analytical protocols

All samples were spiked with appropriate amounts of internal/surrogate standards (PCBs #34, 62, and 119; or $^{13}C_{12}$-BDEs #28 and 47). Hexane extracts were then eluted through a 2 g column of anhydrous sodium sulfate to remove any moisture. After concentration to 250 μL in nonane, they were transferred to a GC vial and spiked with appropriate amounts of recovery determination standard (PCB #129).

All analyses were carried out on a Fison's GC8000/MD800 GC-MS fitted with an SGE BP-5 capillary column (60 m × 0.25 mm id × 0.25 μm film thickness), running temperature programs of: (a) for PCBs 140 °C for 2 min, 5 °C min^{-1} to 200 °C, and 2 °C min^{-1} to 280 °C; and (b) for PBDEs 140 °C with 5 °C min^{-1} ramp to 200 °C, and 2 °C min^{-1} to 300 °C, held for 10 min. Both the injector and interface were held at 280 °C. Helium was used as the carrier gas at a head pressure of 180 kPa. One μl of each sample was injected in splitless mode. Initial screening of sample extracts running the mass spectrometer in full scan mode (m/z 50–650) revealed only lower halogenated PCBs or PBDEs or biphenyl (for PCB experiments) to be present. Hence, in all subsequent analyses, the mass spectrometer was operated in SIM mode, targeting the starting compound and its lower halogenated analogues. Monitored m/z values for these analyses were as reported previously,[9,22] with the addition of 154.10 for biphenyl.

Identification of all compounds was achieved by comparison with authentic standards, with quantification relative to the internal standard of the same or nearest homologue group. To ensure accurate and precise measurement of compounds in the selected ion mode, peaks were only accepted if the following criteria were met.

- Signal to noise ratios for the least abundant ion exceeded 3:1.
- Peaks eluted within 5 s of standards run in the same batch as the samples.
- Isotope ratios for peaks were within 20% of those obtained for standards run in the same batch as the samples. This applied to halogenated compounds only.

One analytical blank composed of clean MOPS buffer was run for every 5 samples. None of the target compounds were detected in the analytical blanks. Recoveries of internal/surrogate standards in all samples and blanks ranged between 75 and 100%.

Results and discussion

PCB exposure experiments

Although determination of the relative abundance of products at different exposure times is needed to confirm this, the relative abundance of the products of these 24 h exposure experiments (Table 1) suggests that the Bio-Pd^0 causes PCBs to undergo dechlorination in a stepwise fashion. For example, the trichlorinated PCB #28 (2,4,4′-trichlorobiphenyl) is first dechlorinated to yield the following dichlorinated congeners in order of preferential formation: 2,4′-dichlorobiphenyl (PCB #8—loss of *p*-chlorine), 2,4-dichlorobiphenyl (PCB #7—loss of *p*-chlorine), and 4,4′-dichlorobiphenyl (PCB #15—loss of *o*-chlorine). Further dechlorination to the monochlorinated congeners 2-chlorobiphenyl (PCB #1) (most abundant, arising from loss of *p*-chlorine from the most abundant dichlorobiphenyl products) and 4-chlorobiphenyl (PCB #3—loss of

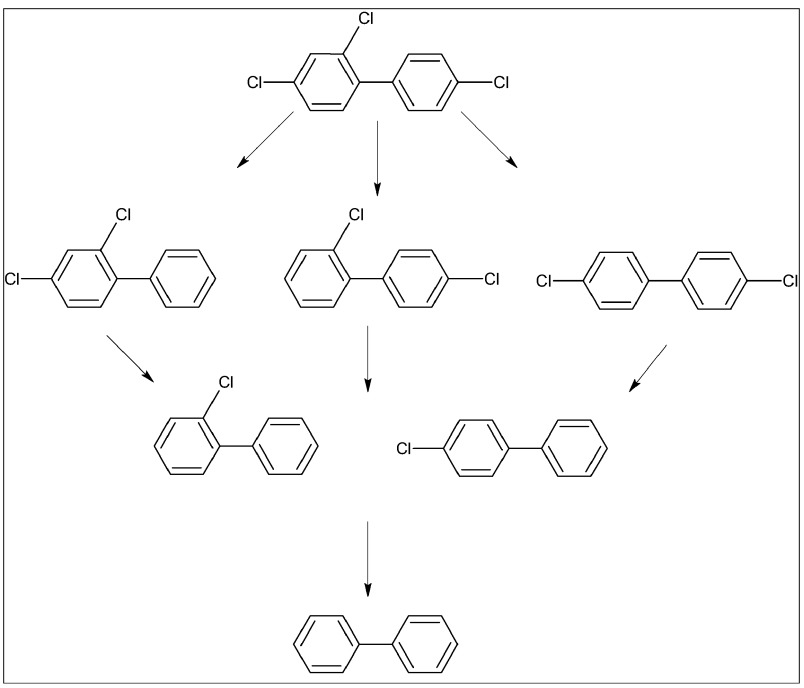

Fig. 1 Dechlorination pathways for PCB #28.

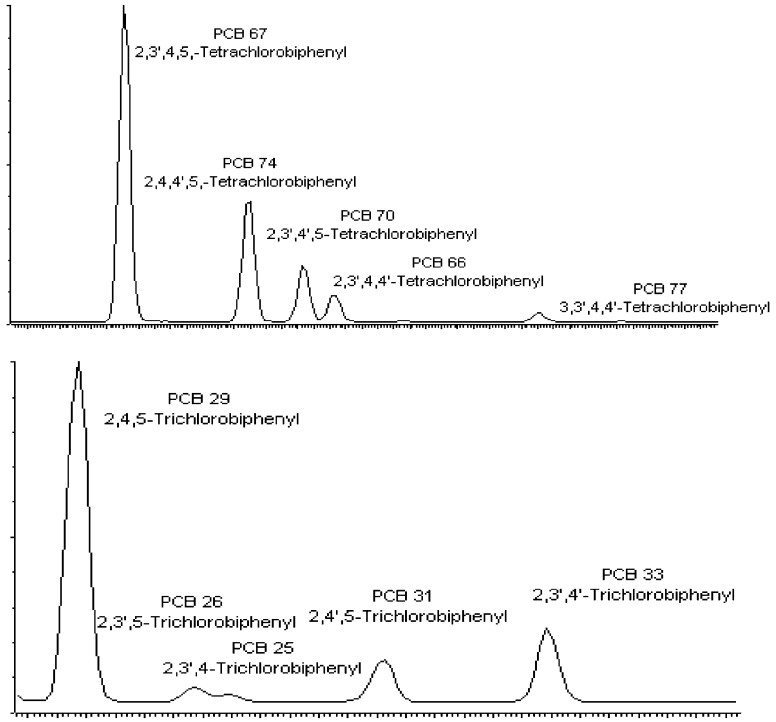

Fig. 2 GC-MS chromatograms showing dechlorination products for PCB #118.

o-chlorine from one of the most abundant dichlorobiphenyl products, and loss of *p*-chlorine from the least abundant dichlorobiphenyl products) occurs, before proceeding to biphenyl (Table 1 and Fig. 1), which appears to be the final product. Overall, a mass balance calculation for PCB #28 reveals that after 24 hours exposure to Bio-Pd^0, around 70% of the initial challenged mass (Table 1) was degraded, with around 30% of the starting material remaining and biphenyl the most abundant product. More extended incubations were not performed.

Our results are in line with those reported recently by De Windt *et al.*[23] These authors challenged 2,3,4-trichlorobiphenyl (PCB #21) with palladized *Shewanella oneidensis* MR-1 at a Bio-Pd concentration of 50 mg L^{-1} in the presence of H_2 as electron donor at 28 °C. Under these conditions, these authors reported 90% removal of PCB #21 within 5 h, and identified as dechlorination products both 2,3-dichlorobiphenyl (PCB #5) and 2-chlorobiphenyl (PCB #1). Other studies reported rapid removal of almost all of the starting PCB compound (V. S. Baxter-Plant, unpublished work), attributed to rapid sorption of PCB molecules onto the palladium nanoparticles within 15 min (I. P. Mikheenko, unpublished work[24]), which prompted analysis of both the residual solution and also hexane extracts of the residual catalyst in the present study.

Challenge of the pentachlorinated PCB #118 revealed similar reductive dechlorination to yield five tetrachlorinated and five trichlorinated PCB products (Table 1 and Fig. 2). No di- or monochlorobiphenyls or biphenyl were identified in these samples. The overall efficiency of dehalogenation of PCB #118 after 24 h was much lower (total yield of identified products was 0.3%); it appears that dehalogenation of this more chlorinated starting material occurs much more slowly than is observed for the trichlorinated PCB #28, possibly attributable to steric hindrances, but this was not tested further.

PBDE #47 exposure experiments

This experiment revealed that PBDE #47 underwent debromination when challenged with the Bio-Pd^0. As with the PCBs, the favoured debromination products were those involving loss of the least hindered bromine atom. A mass balance calculation revealed that while 75% of the starting material remained intact, 10% was converted to identifiable lower brominated PBDEs, predominantly the tribrominated PBDE #17 and the dibrominated PBDE #4, with the remaining *ca.* 15% of starting mass unaccounted for. Given that no diphenyl ether was detected, the unaccountable mass was presumably converted to products resulting from cleavage of the ether bond. Table 1 shows the percentage yields for each congener identified. While these data show the technique to require optimisation in order to achieve its full potential for this class of compounds, these results suggest the potential of this method to treat PBDE-containing wastes.

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